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## Product Information

### SYBR® GREEN II

Product No. **S 9305**

Store at -20 °C

[172827-25-7]

#### Product Description

SYBR® Green II is a highly sensitive stain for post-electrophoresis staining of RNA and ssDNA in agarose or polyacrylamide gels. The detection limit is 500 picograms of RNA per band in non-denaturing gels with 300 nm transillumination (down to 100 pg with 254 nm epi-illumination).<sup>1,2</sup>

On denaturing agarose/formaldehyde gels and polyacrylamide/urea gels, the sensitivity of SYBR Green II RNA gel stain is reduced, though still superior to that of ethidium bromide. Without any washing or destaining steps, SYBR Green II can detect as little as 1 ng RNA per band in agarose/formaldehyde gel or polyacrylamide/urea gel using 254 nm epi-illumination, and about 4 ng RNA per band using 300 nm trans illumination. Staining agarose/formaldehyde gels with SYBR Green II does not interfere with the transfer of RNA to membranes or subsequent hybridization in Northern blot analysis as long as 0.1%-0.3% SDS is included in prehybridization and hybridization buffers to remove the dye.

SYBR Green II is not selective for RNA staining but does exhibit a higher quantum yield when bound to RNA (~0.54) than to double-stranded DNA (~0.36). The fluorescence quantum yield of the RNA/SYBR Green II complex is over seven times greater than that of the RNA/ethidium bromide complex (~0.07).<sup>3</sup> SYBR Green II 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.

SYBR Green II should facilitate the detection of viroid RNAs and multicopy cellular RNA species, and may also prove useful in applications, such as single-strand conformation polymorphism (SSCP) analysis,<sup>4</sup> that require extremely sensitive detection techniques.

Conventional SSCP analysis requires radioactive hybridization probes. Although nonisotopic techniques for SSCP analysis have been developed,<sup>5-8</sup> they require long and complex procedures, such as silver staining or chemiluminescence-detection. Ethidium bromide has been used for nonisotopic SSCP in precast polyacrylamide minigels.<sup>9</sup> Not only is this new nonisotopic SSCP technique simple, rapid and reproducible, but it allows precise temperature control, an important parameter in SSCP analysis. SYBR Green II RNA gel stain should prove more sensitive than ethidium bromide in such applications. SYBR Green II may be used with commonly available ultraviolet epi- and transilluminator excitation sources, as well as hand-held ultraviolet lamps.

#### Components

Supplied as 10,000X solution in DMSO (dimethyl sulfoxide). 1 ml prepares 10 liters of staining solution, sufficient for 100 mini-gels.

#### Precautions and Disclaimer

SYBR Green II is for laboratory use only, not for drug, household or other uses. Refer to the Material Safety Data Sheet (MSDS).

#### Storage/Stability

Store 10,000X SYBR Green II solution at -20 °C. This DMSO stock solution is stable for six months to one year. Diluted staining reagent may be stored protected from light either at 2-8 °C for several weeks or at room temperature for 3-4 days. Staining solutions prepared in water are less stable than those prepared in buffer and must be used within 24 hours

## Procedure

### Staining:

Perform electrophoresis according to standard procedures.<sup>10</sup> Perform staining at room temperature and protect staining solution from light. Staining solutions should be prepared in plastic rather than glass, as the stain may adsorb to glass surfaces. Before opening, allow the vial of SYBR Green II to warm to room temperature and then briefly centrifuge to collect at the bottom of the vial.

1. Prepare a 1:10,000 dilution in 1X TBE buffer (89 mM Tris base, 89 mM boric acid, 1 mM EDTA, pH 8.0). For denaturing agarose/formaldehyde gels, make a 1:5,000 dilution in 1X TBE. The buffer should not have been used previously for electrophoresis for optimal results.  
Note: For optimal sensitivity, verify that the pH is between 7.5 and 8.0 (preferably pH 8.0) as SYBR Green II is pH sensitive.
2. Place the gel in a plastic staining container. 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. The fluorescence of SYBR Green II complexes is not quenched in the presence of urea or formaldehyde
3. 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 at 2-8 °C in the dark and reused three to four times.  
Note: SYBR Green II does not interfere with the transfer of RNA to membranes or subsequent hybridization in Northern blot analysis as long as 0.1%-0.3% SDS is included in prehybridization and hybridization buffers to remove the dye.
4. Solutions of SYBR Green II should be poured through activated charcoal before disposal. One gram of activated charcoal can absorb the dye from 10 liters of freshly prepared working solution. The charcoal may then be disposed of as solid hazardous waste.

### Visualizing and Photographing Stained Gels:

1. Illuminate the stained gel using 300 nm ultraviolet transillumination, or for greater sensitivity, 254 nm epi-illumination (analogous to epi-fluorescence microscopy).
2. Photograph the gel with Polaroid 667 black and white print film (Product No. F 4638) using a Kodak Wratten 15 filter (Product No. F 8390). . If the transilluminator light is especially intense, a UV-blocking filter such as Kodak Wratten 2A, (Product No. F 9265) may be used in conjunction with the Wratten 15 filter to prevent "fogging" or increased background in the photograph. A
3. number of other yellow or green gelatin or cellophane filters can also be used for photography, but most will provide slightly reduced sensitivity. The orange-red filters used to photograph ethidium bromide stained gels are not recommended for visualizing SYBR Green II stained gels as they give poor results.

### Notes:

- a. SYBR Green II has a low intrinsic fluorescence so there is no need to remove free dye. The 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.
- b. Video cameras and CCD cameras in general have a different spectral response than black and white print film and may not exhibit the same sensitivity.

### References

1. FASEB J., **8**, A1266 (1994)
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3. Cytometry, **7**, 508 (1986)
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7. Nucleic Acids Res., **19**, 2500 (1991)
8. Nucleic Acids Res., **19**, 3154 (1991)
9. Nucleic Acids Res., **21**, 3637 (1993)
10. 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)

**Related Products**

- 10X TBE Buffer, Product No. T 4415, diluted to 1X and adjusted to pH 8.0 w/ HCl for SYBR Green use
- Kodak Wratten No. 15 Filter, Product No. F 8390
- Polaroid Type 667 black and white print film, Product No. F 4638
- Precast Agarose Gel for RNA, 1.25%, 8 well, Product No. P 6222
- Acrylamide for Mutation Detection, A high-resolution gel matrix specifically formulated to separate DNA based on conformational differences that are not resolved on conventional polyacrylamide gels, Product No. A 5934

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