SYBR® Green I Nucleic Acid Gel Stain

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
SYBR® Green I nucleic acid gel stain (S7563)	500 µL	10,000X	• ≤–20°C • Desiccate • Protect from light	When stored as directed in DMSO, stain is stable for 6 months to 1 year.
SYBR® Green I nucleic acid gel stain (S7567)	1 mL			
SYBR® Green I nucleic acid gel stain (S7585)	20 vials, 50 µL each			
SYBR® Green Nucleic Acid Gel Stain Starter Kit (S7580)	50 µL each, SYBR® Green I and SYBR® Green II stain and a SYBR® photographic filter			
Number of labelings: 500 µL size is sufficient to stain ~100 minigels. 1 mL size is sufficient to stain ~200 minigels.				
Approximate fluorescence excitation/emission maxima: 290, 380, 497/520 nm, bound to DNA				

Introduction

Molecular Probes SYBR® Green I nucleic acid gel stain is one of the most sensitive stains available for detecting double-stranded DNA (dsDNA) in agarose and polyacrylamide gels. With 300 nm transillumination, as little as 60 pg dsDNA per band can be detected with SYBR® Green I stain. Less than 20 pg of dsDNA can be detected in a single band of a SYBR® Green I–stained gel using 254 nm epi-illumination, black-and-white Polaroid 667 print film, and a SYBR® photographic filter (S7569).[1] This sensitivity is at least 25 times greater than can be achieved with ethidium bromide using standard 300 nm transillumination (Figure 1). We also have found that SYBR® Green I stain is also a very sensitive stain for oligonucleotides, allowing the detection of as little as 1–2 ng of a synthetic 24-mer on 5% polyacrylamide gels—a sensitivity that is 50–100 times greater than can be achieved with ethidium bromide.[2]

The sensitivity for detecting single-stranded DNA and RNA is somewhat lower (approximately 100 to 300 pg per band using 254 nm epi-illumination), making SYBR® Green I stain ideal for detecting dsDNA in complex solutions, where ssDNA or RNA in the sample may obscure the results, such as apoptosis ladders.[3]

The remarkable sensitivity of SYBR® Green I stain for detecting nucleic acids in gels can be attributed to a combination of unique dye characteristics. SYBR® Green I stain exhibits exceptional affinity for DNA and a large fluorescence enhancement upon DNA binding—at least an order-of-magnitude greater than that of ethidium bromide. Also, the fluorescence quantum yield of the DNA/SYBR® Green I complex (~0.8) is over five times greater than that of DNA/ethidium bromide (~0.15). SYBR® Green I stain quickly penetrates both agarose and

polyacrylamide gels and has negligible background fluorescence in the absence of DNA, allowing a rapid staining procedure that requires no destaining step prior to photography.

The exceptional sensitivity of SYBR® Green I stain makes it useful for many applications where the amount of DNA is limiting, including the detection of low–cycle number and low–target number DNA amplification products;[4] the detection and restriction analysis of low–copy number DNA and RNA vectors and of cosmids, plasmids, and phage DNA from cultures with low cell numbers; and the detection of products of nuclease protection and bandshift assays. SYBR® Green I stain's superior sensitivity has even allowed the replacement of radioisotopes in some applications.[5-8]

Because of SYBR® Green I stain's strong DNA binding affinity, it can be used to stain DNA before electrophoresis (prestaining), as well as after electrophoresis (poststaining),[9] and has also been used to stain DNA separated by capillary electrophoresis.[10,11] Gels can be precast with SYBR® Green I stain; however, we have found the greatest sensitivity is achieved by poststaining. Furthermore, our researchers have found that the presence of SYBR® Green I stain bound to DNA does not inhibit the activity of many common restriction endonucleases, including *Hin*d III and *EcoR* I [1,2] and does not interfere with Southern blotting techniques.

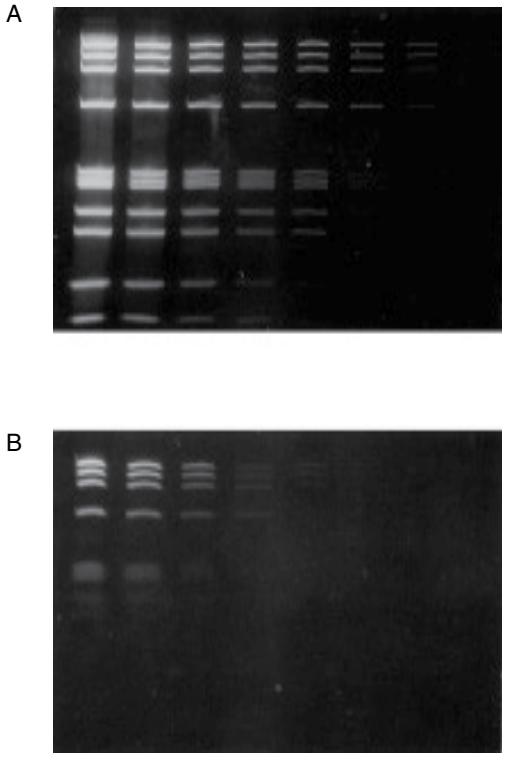

Figure 1. Dilution series of φX174 RF DNA cut with *Hae*III restriction endonuclease and electrophoresed in 5% polyacrylamide gels. The gels contain an identical threefold dilution series of DNA fragments. The gel shown in panel A was stained for 30 minutes with SYBR® Green I nucleic acid gel stain (using a 1:10,000 dilution of the stock reagent) and not destained. The gel shown in panel B was stained with 5 µg/mL ethidium bromide for 30 minutes, then destained for 30 minutes. The SYBR® Green I stained gel was excited using 254 nm epi-illumination and the ethidium bromidestained gel using 300 nm transillumination (Fotodyne Foto/UV 450 ultraviolet transilluminator). Although SYBR® Green I dye stained gels 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 I stained gel) or an ethidium bromide gel stain photographic filter (ethidium bromide–stained gel).

Before You Begin

Before opening, the vial must be warmed completely to room temperature to ensure that the DMSO is completely thawed and that the solution is homogeneous. To avoid losing stain, briefly centrifuge thawed stain in a microfuge to deposit the DMSO solution at the bottom of the vial. Stain may be divided into smaller aliquots and frozen for convenience. Smaller aliquots will thaw more quickly.

Materials Required but Not Provided

- TE, TBE, or TAE buffer
- Optional: ethanol for precipitating DNA during SYBR® Green I dye removal

Handling and Disposal

Ames testing by an independent laboratory has shown that SYBR® Green I nucleic acid gel stain is significantly less mutagenic than ethidium bromide.[12] **However, we must caution** that no data are available addressing the mutagenicity or toxicity of SYBR® Green I stain in humans. 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. Dispose of the stain in compliance with local regulations.

Spectral Characteristics

SYBR® Green I stain is maximally excited at 497 nm, but also has secondary excitation peaks at ~290 nm and ~380 nm (Figure 2). The fluorescence emission of SYBR® Green I stain bound to DNA is centered at 520 nm. These spectral characteristics make SYBR® Green I 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.

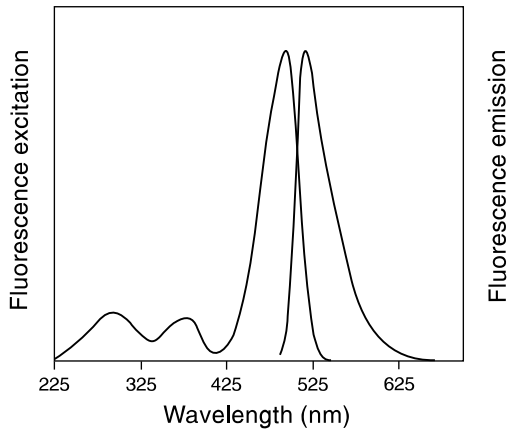

Figure 2. Fluorescence excitation and emission spectra of dsDNA-bound SYBR® Green I nucleic acid gel stain.

Experimental Protocols

Staining DNA Following Electrophoresis

1. **Perform electrophoresis on an agarose or nondenaturing polyacrylamide gel.**

   - TBE (89 mM Tris base, 89 mM boric acid, 1 mM EDTA, pH 8) and TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8) buffers are both compatible with SYBR® Green I staining.

2. **Dilute the stock SYBR® Green I reagent 1:10,000.**

   - Stain may be diluted in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), TBE, or TAE buffer.
   - Staining with SYBR® Green I 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).
   - 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 about 7.5 or above 8.0 are less stable and show reduced staining efficacy.

3. **Cover the gel with staining solution and incubate at room temperature for 10–40 minutes.**

   - Use a plastic container, such as a Rubbermaid Servin' Saver sandwich box or the top of a pipet-tip box. Do not use a glass container, as it will adsorb much of the dye in the staining solution.
   - Protect the staining container from light by covering it with aluminum foil or placing it in the dark.
   - Agitate the gel gently at room temperature.
   - Staining time will 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) for a week or more and reused up to four times.
   - We recommend storing aqueous stain solutions in plastic rather than glass, as the stain may adsorb to glass surfaces.

Precasting SYBR® Green I Gels

Precast agarose or nondenaturing polyacrylamide gels with SYBR® Green I stain by diluting the SYBR® Green I stock reagent 1:10,000 into the gel solution just prior to pouring the gel. The DNA detection limit for gels precast with SYBR® Green I stain may be slightly higher (on the order of 30 to 40 pg/band) than for gels stained after electrophoresis (less than 20 pg/band). In addition, the rate of migration of DNA fragments in SYBR® Green I gels may be significantly slower than the rate of migration of the same fragments in a gel containing no dye.

Staining DNA Before Electrophoresis

In general, DNA is incubated with a 1:10,000 dilution of the dye (in TE, TBE, or TAE) for at least 15 minutes prior to electrophoresis. We have stained 1 µg of molecular weight–marker DNA with a 1:10,000 dilution of SYBR® Green I stain in a total volume of 16 µL. SYBR® Green I stain has not yet been tested as a prestaining label for DNA templates in band-shift assays, although it may prove useful in this application. See references 4 and 5 for general methods on how to stain DNA before electrophoresis. It may be necessary to optimize the protocols in these references for the specific application.

Photographing the Gel

UV or blue-light transillumination or UV epi-illumination

DNA stained with SYBR® Green I stain can be readily visualized using a UV or blue-light sources.

- Epi-illumination with 254 nm will give a higher sensitivity than 300 nm transillumination. It is important to clean the surface of the transilluminator after each use with deionized water and a soft cloth (like cheesecloth). Otherwise, fluorescent dyes, such as SYPRO® stains, SYBR® stains, and ethidium bromide, will accumulate on the glass surface and cause a high background fluorescence.
- For optimal sensitivity with Polaroid 667 black-and-white film, SYBR® Green I–stained gels should be photographed through a Molecular Probes SYBR® photographic filter (S7569). 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 red/orange filters used to photograph ethidium bromide–stained gels, should not be used with SYBR® Green I–stained gels. For filters to use with hand-held cameras or CCD cameras, contact your instrument manufacturer.
- Stained gels have negligible background fluorescence, allowing long film exposures when detecting small amounts of DNA. For 300 nm transillumination, we typically take a 1–2 second exposure using an F-stop of 4.5 on a typical Polaroid camera. 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.
- Our detection limits are based on results obtained with a FOTODyne FOTO/UV 450 ultraviolet transilluminator in combination with Polaroid 667 black-and-white print 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.

Laser-scanning instruments

SYBR® Green I stained DNA can be visualized using imaging systems equipped with lasers that emit at 450, 473, 488, or 532 nm. Please contact the manufacturer of your instrument for optimal filter sets to use with SYBR® Green I stain.

Removing SYBR® Green I from Double-Stranded DNA

We have found that more than 99% of SYBR® Green I stain can be removed from double-stranded DNA by simple ethanol precipitation. Bring a solution of SYBR® Green I–stained DNA up to 100 mM NaCl and add 2½ volumes of absolute or 95% ethanol. After incubating for about 20 minutes on ice, centrifuge mixture for at least 10 minutes in a microfuge at 4°C. Remove the ethanol and wash the pellet once with 70% ethanol. Evaporate the ethanol and resuspend double-stranded DNA in TE.

References

1. Biophys J 66, A159 (1994); **2.** Biomed Products 19, 68 (1994); **3.** Proc Natl Acad Sci USA 94, 12419 (1997); **4.** J Virol Methods 55, 153 (1995); **5.** FASEB J 10, A1128, abstract #751 (1996); **6.** Methods Cell Sci 17, 1 (1995); **7.** Biotechniques 23, 1029 (1997); **8.** Nucleic Acids Res 25, 2945 (1997); **9.** Methods Enzymol 217, 414 (1993); **10.** Clin Chem 43, 267 (1997); **11.** Biotechniques 22, 1107 (1997); **12.** Mutat Res 439, 37 (1999).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
S7563	SYBR® Green I nucleic acid gel stain *10,000X concentrate in DMSO*	500 µL
S7567	SYBR® Green I nucleic acid gel stain *10,000X concentrate in DMSO*	1 mL
S7585	SYBR® Green I nucleic acid gel stain *10,000X concentrate in DMSO* *special packaging*	20 x 50 µL
S7569	SYBR® photographic filter	each
S7580	SYBR® Green Nucleic Acid Gel Stain Starter Kit	1 kit

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