SYBR® Gold Nucleic Acid Gel Stain

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
SYBR® Gold nucleic acid gel stain	500 µL	Solution in high-quality, anhydrous DMSO *	• ≤–20°C • Desiccate • Protect from light	When stored as directed, stain stock solution is stable for 6 months to 1 year.

* DMSO stock solution is a 10,000X concentrate.

Number of labelings: Sufficient dye is provided to stain at least 100 agarose or polyacrylamide minigels.

Approximate fluorescence excitation/emission maxima: 300, 495/537 nm, bound to nucleic acid

Introduction

Molecular Probes SYBR® Gold nucleic acid gel stain is the most sensitive fluorescent stain available for detecting double- or single-stranded DNA or RNA in electrophoretic gels, using standard ultraviolet transilluminators—surpassing even the sensitivity of our SYBR® Green gel stains in this application.[1] SYBR® Gold stain is a proprietary unsymmetrical cyanine dye that exhibits >1000-fold fluorescence enhancement upon binding to nucleic acids and has a high quantum yield (~0.6) upon binding to double- or single-stranded DNA or to RNA.[1] Excitation maxima for dye–nucleic acid complexes are at ~495 nm in the visible and ~300 nm, in the ultraviolet (Figure 1). The emission maximum is ~537 nm. SYBR® Gold stain is >10-fold more sensitive than ethidium bromide for detecting DNA and RNA in denaturing urea, glyoxal, and formaldehyde gels, even with 300 nm transillumination.[1] For detecting glyoxalated RNA, SYBR® Gold stain is 25–100 times more sensitive than ethidium bromide (Figure 2) and is by far the most sensitive stain available for this application.[1] SYBR® Gold stain has also been shown to be much more sensitive than SYBR® Green II stain for detecting single strand conformation polymorphism (SSCP) products.[2] SYBR® Gold stain penetrates thick and high percentage agarose gels rapidly, and even formaldehyde agarose gels do not require destaining, due to the low intrinsic fluorescence of the unbound dye. The presence of the dye in stained gels at standard staining concentrations does not interfere with restriction endonucleases, T4 DNA ligase, Taq polymerase, or with Southern or Northern blotting.[1] Dye is readily removed from nucleic acids by ethanol precipitation, leaving pure templates available for subsequent manipulation or analysis.

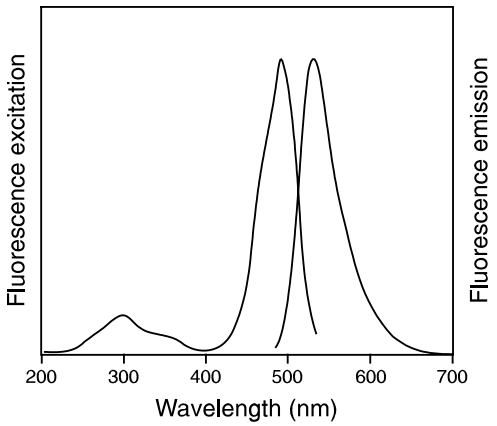

Figure 1. Excitation and emission spectra of SYBR® Gold nucleic acid gel stain bound to double-stranded DNA.

Before You Begin

Materials Required but Not Provided

- TE, TBE, or TAE buffer
- SYBR® photographic filter (S7569)
- Ethanol
- Sodium acetate or ammonium acetate

Working with the SYBR® Gold Gel Stain

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. Be sure the dye solution is fully thawed before removing an aliquot.

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 about 7.0 or above 8.5 are less stable and show reduced staining efficacy. We recommend storing aqueous working solutions in plastic rather than glass, as the stain may adsorb to glass surfaces.

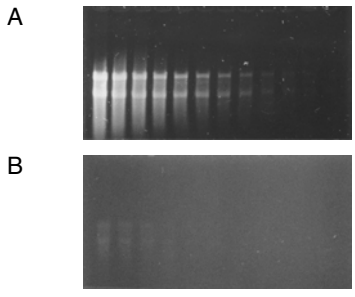

Figure 2. Comparison of glyoxalated RNA stained with SYBR® Gold stain and with ethidium bromide. Identical twofold dilutions of glyoxalated *E. coli* 16S and 23S ribosomal RNA were separated on 1% agarose minigels using standard methods [2] and stained for 30 minutes with SYBR® Gold stain in TBE buffer (A) or 0.5 µg/mL ethidium bromide in 0.1 M ammonium acetate (B). Both gels were subjected to 300 nm transillumination and photographed with Polaroid 667 black-and-white print film, through a SYBR® photographic filter (S7569) for the gel stained with SYBR® Gold dye and through an ethidium bromide gel stain photographic filter for the gel stained with ethidium bromide.

Caution

No data are available addressing the mutagenicity or toxicity of SYBR® Gold nucleic acid gel stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled 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.

Disposal

As with all nucleic acid reagents, solutions of SYBR® Gold stain should be disposed of in accordance with local regulations.

Experimental Protocol

The protocol below describes how to stain minigels with SYBR® Gold stain after electrophoresis. To stain agarose gels or polyacrylamide minigels, immerse the entire gel in staining solution. To stain large or extremely fragile polyacrylamide gels, leaving the gel on one of the gel plates and overlaying the gel with dye is probably a more practical procedure. When employing the dye overlay procedure, be sure to turn the stained gel upside down on the transilluminator prior to photography, as most glass plates will block at least some of the ultraviolet light, resulting in poor excitation of dye–nucleic acid complexes. Casting gels containing SYBR® Gold stain is not recommended, as the dye causes severe electrophoretic mobility retardation of nucleic acids in the gel.

Staining Minigels with SYBR® Gold Stain

1.1 Dilute the stock SYBR® Gold stain 10,000-fold to make a 1X staining solution.

- Dilute into TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5–8.0), TBE (89 mM Tris base, 89 mM boric acid, 1 mM EDTA, pH 8.0), or TAE (40 mM Tris-acetate, 1 mM EDTA, pH 7.5–8.0) buffer.
- Staining with SYBR® Gold stain is somewhat pH sensitive. For optimal sensitivity, verify that the pH of the staining solution at the temperature used for staining is between 7.0 and 8.5.

1.2 Incubate the gel in 1X staining solution for 10–40 minutes.

- Place the gel in the staining container, such as a petri dish, the lid of a pipet-tip box, or a polypropylene container.
- Add enough staining solution to completely cover the gel. A 50 mL volume is generally sufficient for staining most standard minigels. To stain large agarose gels, scale up the volume of staining solution in proportion to the increased gel volume and ensure that the entire gel is fully immersed during staining.
- Protect the staining solution from light by covering it with aluminum foil or by placing it in the dark.
- Prewashes of gels are not required, even for gels containing urea, formaldehyde, or glyoxalated samples. Removal of the glyoxal is also not necessary.

1.3 Agitate the gel gently at room temperature.

- The optimal staining time is typically 10-40 minutes, 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 and can be reused 3–4 times, although best results are obtained from fresh staining solution.

Viewing and Photographing the Gel

2.1 Illuminate the stained gel.

- Blue-light transilluminators, such as Invitrogen's Safe Imager™ blue-light transilluminator also show excellent sensitivity with SYBR® Gold stained gels.
- Stained gels may also be viewed with 300 nm ultraviolet or 254 nm epi- or transillumination.
- Stained gels may also be visualized and anaylzed with laser scanners. Maximum visible-light excitation is 495 nm.

2.2 Photograph the gel.

- Gels may be photographed using Polaroid 667 black-and-white print film and a SYBR® photographic filter (S7569). When using Polaroid film and this filter, we find that when exciting gels at 300 nm using the FOTO/UV® 450 transilluminator (FotoDyne, Inc., Hartland, WI), a 0.5-1.0 second exposure with an f-stop of 5.6 is generally optimal. Optimal photographic conditions should be determined empirically for other light sources.
- With 254 nm epi-illumination, exposures of ~1 minute may be required for maximal sensitivity when using Polaroid film and the SYBR® filter.
- Generally, optimal exposure times for SYBR® Gold dye-stained gels are shorter than those required for identical gels stained with the SYBR® Green gel stains, due to the higher quantum yield of SYBR® Gold stain.
- Gels stained with the SYBR® Gold dye can also be documented using CCD cameras or laser scanner systems equipped with appropriate optical filters. Generally filters designed for use with the SYBR® Green gel stains are adequate. Optimal exposure times or other instrument settings will have to be determined empirically.

Removing SYBR® Gold Stain from Nucleic Acids

The SYBR® Gold stain can be efficiently removed from nucleic acids by simply precipitating the DNA or RNA with ethanol. More than 97% of the dye is removed by a single precipitation step. More than 99% of the dye is removed if ammonium acetate is used as the salt in the precipitation procedure.

3.1 Add one of the following salts to the nucleic acid sample, to the indicated final concentration: 200 mM NaCl, 300 mM sodium acetate (pH 5.2) or 2.0 M ammonium acetate. Mix gently.

3.2 Add two volumes of ice-cold absolute ethanol and mix well. Incubate at 0°C (on ice) for 30 minutes.

3.3 Pellet nucleic acids by centrifuging for at least 15 minutes at 10,000–12,000 × g.

3.4 Remove the supernatant and wash the pellet with 70% ethanol.

3.5 Centrifuge again to pellet nucleic acids.

3.6 Allow the pellet to air dry and resuspend as desired.

References

1. Anal Biochem 268, 278 (1999); **2.** Personal communication, Chris Weghorst, Ohio State Universtity; **3.** Proc Natl Acad Sci USA 74, 4835 (1977).

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

Cat #	Product Name	Unit Size
S11494	SYBR® Gold nucleic acid gel stain *10,000X concentrate in DMSO*	500 µL
S7569	SYBR® photographic filter	each

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