SYBR Nucleic Acid Gel Stains

Introduction

Since their introduction in late 1993, our SYBR® nucleic acid gel stains have rapidly become very popular due to their high sensitivity and ease of use. However, as with any new technology, there has been much to learn. The basic information on the use of each SYBR dye is contained in the specific *Product Information Sheet* provided with each dye. This *Useful Tips* publication elaborates on the basic information to help you get the most out of these remarkable reagents. The tips are organized into sections that focus on specific topics. In this document, "SYBR stain" refers to either SYBR Gold, SYBR Green I or SYBR Green II gel stain. Any notable differences are indicated.

Storage and Handling

1.1 Stock Solutions

SYBR stains are supplied as stock solutions in DMSO. We have found these dyes are extremely stable to repeated freeze/thaw cycles. We recommend that you protect them from light as much as possible, particularly once they have been diluted for use. We also recommend dividing the stock solutions of dye into aliquots for convenience or purchasing the 20 × 50 µL special packaging. Smaller aliquots will thaw faster.

1.2 Concentration Changes in DMSO Stock Solutions

Use of stock solutions that are incompletely thawed can result in loss of staining efficiency over time. When the stock solution is thawed, dye is more concentrated in the first volumes of liquid DMSO. Subsequent rounds of incomplete thawing will further lower the concentration of the remaining stock. It is therefore essential that the whole volume be allowed to thaw and then mixed thoroughly before any portions are removed. Dividing the dye solution into aliquots for storage will greatly speed the thawing process.

1.3 Storage Containers

Polypropylene plastics (marked with the recycle number 5) seem to bind the least dye, whereas glass appears to bind the most dye. Solutions containing SYBR stains should not be left in contact with glass. Even with plastics, however, there will be a period of time during which the dye will gradually saturate the plastic. Until the plastic is saturated with the dye, you may find the solution gives poor staining after storage. Because of this phenomenon, we recommend that you have staining dishes and storage containers reserved exclusively for the SYBR stains. Our favorite SYBR dye storage containers are the Rubbermaid Servin' Saver® sandwich boxes.

1.4 Shelf Life of Working Solutions

The stability of the working solution during storage depends upon the type of container used (see section 1.3, *Storage Containers*), buffer system (see *Staining Issues*, below) and storage conditions. Working solution prepared in the recommended manner can be stored in an airtight, light-protected (foil wrapped or dark) polypropylene container in the refrigerator between uses. Under these conditions, working solutions will reliably last 6 or 7 days.

1.5 Reusing Working Solutions

We have had varying reports about the number of times a working solution of SYBR stain can be reused. The actual number of gels that can be stained will depend on the size and type of gel, the amount of nucleic acid in the gel, as well as the amount of time between staining sessions (see section 1.4, *Shelf Life of Working Solutions*). Several minigels can be stained with one batch of staining solution on the day it is prepared. Larger or thicker gels soak up more dye and require a larger volume of staining solution, and may also limit the reuse of the staining solution.

1.6 Handling

The SYBR stains are nucleic acid binding reagents and as such should be considered potential carcinogens. Furthermore, DMSO is a very effective carrying agent and will quite readily permeabilize tissue, allowing the dye to enter. We strongly recommend that SYBR stains always be handled with the same care given to other nucleic acid stains, such as ethidium bromide.

1.7 Mutagenicity

An independent laboratory showed SYBR Green I nucleic acid gel stain to be significantly less mutagenic than ethidium bromide in the Ames test.[1]

1.8 Disposal

We have found an effective method for disposal of SYBR stains is to filter the staining solutions through activated charcoal. The charcoal can then be disposed of by incineration. One gram of activated charcoal easily absorbs the dye from 10 liters of freshly prepared working solution.

Staining Issues

2.1 Buffers

The SYBR dyes must be diluted into a buffered solution; TAE, TE or TBE buffers are fine, though pH is a consideration

(see section 2.2). The dyes are not stable in water alone. All staining solutions must be protected from light and should be stored in the refrigerator at 4°C between uses. Old or used electrophoresis buffer often gives poor staining; we recommend that you always use freshly prepared buffer.

2.2 Important pH Considerations

Check the pH of the staining solution after it is completely prepared and at the temperature at which you plan to do the staining. We have found that the pH can have a major effect on the staining efficiency. If it is greater than 8.3 or less than 7.5 you will see a significant drop in sensitivity. If you plan to refrigerate your staining solution, we recommend that it be pH 7.5 at room temperature (see next section).

2.3 Tris in the Refrigerator

One major consideration when storing working solutions of SYBR stains is that there is a significant increase in the pH of Tris buffers when stored at 4°C versus room temperature. If your buffers were prepared at pH 8.0 at room temperature, then the pH will increase to 8.5 at 4°C. This increased pH is beyond the range at which SYBR stains are most stable.

2.4 Detergents

Do not add any SDS to the electrophoresis buffer as this will dramatically reduce staining efficiency. Triton® X-100 does not have any noticeable effect. The staining dishes should not be rinsed with detergent solutions, as this will affect subsequent staining.

2.5 Staining Dishes

Do not use glass staining dishes. Rubbermaid Servin'Saver containers (marked with the recycle number 5) work very well as staining dishes and come with a lid. Lids from the racks of most brands of pipette tips also make excellent staining dishes. Reserve your staining dishes exclusively for use with SYBR stains. Do not wash staining dishes with soap or detergent. Staining dishes will normally take on an orange color with time.

2.6 Double Staining Gels

It is possible to use one of the SYBR stains on a gel that was initially stained with ethidium bromide, though sometimes with reduced sensitivity compared to using the SYBR stain alone. Double staining may allow improved resolution of bands that were only faintly detectable with ethidium bromide alone. Soaking the gel in water or buffer prior to SYBR Green staining to remove some of the unbound ethidium bromide may improve sensitivity.

2.7 DNA and Modified Nucleotides

DNA containing only deaza-G–modified nucleotides in place of guanine have been reported to stain poorly with SYBR Green I nucleic acid gel stain. There appears to be no way around this problem, though it is possible that using a mixture of modified and unmodified G bases would allow staining.

2.8 Fuzzy Bands

A few customers who switched from ethidium bromide to SYBR Green I gel stains, report that their DNA bands now appear "fuzzy." More precisely they have a distinct band with a diffuse staining region just ahead or behind the main band. This may be due to sample degradation, partially denatured DNA, minor PCR product species, PCR template or contaminating RNA. Sample components that were previously undetectable with ethidium bromide become visible with SYBR stains. High concentrations of hydrophobic proteins can be detected by SYBR dyes, so BSA carrier protein present in some restriction digests may appear as a fuzzy band.

Precasting Dye in Gels and Prestaining Samples

3.1 General Prestaining Considerations

The recommended method for using SYBR stains with agarose or polyacrylamide gels is to stain the gel after electrophoresis at a 1:10,000 dilution of the stock solution. Staining formaldehyde agarose RNA gels after electrophoresis may work somewhat better with a 1:5000 dilution of SYBR Green II stain. However, many researchers are accustomed to casting gels containing ethidium bromide dye. SYBR Green stains can be used this way, however, the mobility of the nucleic acids may be affected (see section 3.2, *Dye Concentration in Precast Gels*) and there may be some loss of sensitivity due to increased background fluorescence. When precasting gels, the dye should be added to the melted gel immediately prior to pouring into the mold, when the liquid is as cool as possible. Boiling and near-boiling temperatures destroy the ability of SYBR Green dyes to stain nucleic acids. Do not heat SYBR Green stains in the microwave.

3.2 Dye Concentration in Precast Gels

Our best results have been achieved with a final dilution of SYBR Green stains of 1:10,000 of the stock solution. We have also had reports that a 1:20,000 concentration produces excellent results with reduced background, but gives a somewhat lower signal. The final dilution is best determined empirically, as there may be some effects on the migration of the nucleic acid fragments. We have seen this occur with SYBR Green I stain and the effect is not linear with fragment size. The mobility of smaller molecules tends to be affected more than that of larger fragments.

3.3 Storage of Unused Gels

We do not recommend that you make gels prestained with SYBR stains more than 1–2 days in advance. The SYBR Green stains will degrade during storage under these conditions. If you run such a gel and find the staining has decreased you will still be able to post-stain the gel according to the standard staining protocol.

3.4 Adding Dye to Loading Buffer

We have previously recommended using a final dye concentration of 1:10,000, though the optimal concentration for your particular application is probably best determined empirically. Customer feedback indicates that SYBR Green stains can be added directly to the loading buffer at a final concentration of 1:1000 without any major disruptive effects on the migration of the nucleic acids. In order to effect such a dilution you can first prepare an intermediate 1:100 dilution of the SYBR Green stain in high-quality anhydrous DMSO. The intermediate dilution can be stored in the freezer and reused. Add 1 µL of this dilution to 9–10 µL of your sample before loading. The 10% final DMSO does not appear to affect the running of the gel.

3.5 Slow Band Migration

If you have cast SYBR stains into your gels, you may be tempted to increase your voltage to compensate for the slower migration. We recommend against this, as it can cause localized heating of the gel resulting in aberrant migration of bands or even heat-caused damage to the SYBR dyes.

3.6 Smearing and Distorted Bands

If the dye is cast into the gel, or the nucleic acid prestained during loading, you may see some smearing or distortion of the bands. SYBR Green stains are very sensitive to nucleic acid overloading. We recommend that each lane contain 1–5 ng of nucleic acid per band to avoid this problem. We do not recommend including the dye in the running buffer as it will disrupt the migration of the nucleic acids and cause smearing of the bands.

3.7 Black Gaps in DNA Bands

Black gaps in the DNA bands have been reported by several researchers using gels cast with SYBR Green I nucleic acid gel stain. We speculate that this may be due to DNA overloading in combination with electrophoresing at too high a voltage. The result could be localized heating and disruption of the DNA/dye complex. Another possibility, however, could be self quenching of the dye's fluorescence due to a high local concentration.

Gel Issues

4.1 Commercial Precast Gels

Some commercially available precast gels may be too thick to allow adequate penetration of the dyes. SYBR Gold stain penetrates thick gels better than either SYBR Green stain. Optimal gel thickness is 4 mm or less. You can compensate for thick gels by lengthening the post-staining time or increasing the staining solution volume, but neither of these techniques will result in the same sensitivity seen with thinner gels. You can also try adding the dye to the loading buffer (see section 3.4, *Adding Dye to Loading Buffer*).

4.2 Sequencing Gels

Vertical gels, gels bound to glass plates or GelBond® film must be post-stained by applying the staining solution directly to the gel and keeping it away from the glass as much as possible. Do this by placing the gel face up in a staining dish and gently pouring the staining solution directly onto the gel. You may improve this further by placing a border of adhesive tape around the gel to help retain the dye solution on the upper surface. A small amount of staining solution can be distributed evenly by rolling a glass pipet gently over the gel surface. While the staining solution should not remain in contact with the glass pipet for an extended time, the brief contact necessary for this rolling distribution technique does not interfere with staining.

4.3 Drying Stained Gels

Drying a gel onto a backing paper can make it difficult to visualize on a transilluminator and can increase the background light scatter under epi-illumination. Most commercially available transparent backing/support films are very effective at blocking UV light, significantly reducing the detection of the SYBR dye–stained bands if the film is allowed to block the light source. Be certain that these gels are viewed film-side-up when placed on a transilluminator. At best, the additional background produced by these films decreases sensitivity and makes visualizing bands very difficult. We've been told that BioRad's plastic support does pass UV light and can be used with SYBR dye–stained gels, though we have not confirmed this ourselves.

Blotting, Restriction Analysis and DNA Recovery

5.1 Blotting

SYBR stains are compatible with Southern and Northern blotting procedures. The dyes are retained during electrophoretic and capillary transfer, though there may be some loss during washing. They do not affect transfer efficiency; however, if allowed to remain, they can affect subsequent hybridization. 0.1–0.3% SDS in the prehybridization blot equilibration buffer or hybridization buffer will remove the dye.

5.2 Staining Nucleic Acids on Membranes

The success of this particular application is very dependent on membrane type. HYBOND® membranes and other positively charged nylon membranes give the best results. On such membranes SYBR stains are far superior to ethidium bromide or methylene blue. However, on nitrocellulose and on some neutral nylon membranes the SYBR stains produce a high nonspecific background that dramatically reduces sensitivity.

5.3 Restriction Analysis

We have not observed any inhibition or interference of restriction endonuclease, ligase or Taq polymerase activity when using SYBR stains at the recommended 1:10,000 dilution. Higher dye concentrations do affect enzyme activity.

5.4 DNA Recovery

The SYBR stains can easily be removed from nucleic acids by ethanol precipitation. Isopropyl alcohol precipitation is somewhat less effective at removing dye; butyl alcohol extraction, chloroform extraction and phenol extraction do not remove dye efficiently.

Photography and Detection Systems

6.1 Photography Overview

Photography (or electronic imaging) of SYBR dye–stained gels is essential to visualize small amounts of DNA. The great sensitivity of SYBR stains arises from the fact that the dyes have very low intrinsic fluorescence and a large fluorescence enhancement upon binding nucleic acids. Consequently, the background fluorescence of the dyes in stained gels is very low. The low background permits photographic film to integrate the signal over time.

6.2 Transilluminators

SYBR stains can be used on most standard transilluminators. Figure 1 shows the excitation and emission spectra of SYBR Green I nucleic acid gel stain bound to double-stranded DNA. In addition to the major excitation peak at 497 nm, SYBR Green I stain has secondary excitation peaks in the UV range, at ~290 nm and ~380 nm. Figure 2 shows the excitation and emission–spectra of SYBR Gold nucleic acid gel stain bound to dsDNA. The SYBR Gold stain has a major excitation peak at 495 nm and a secondary excitation peak at ~300 nm. Thus, commonly used UV light sources are suitable for the

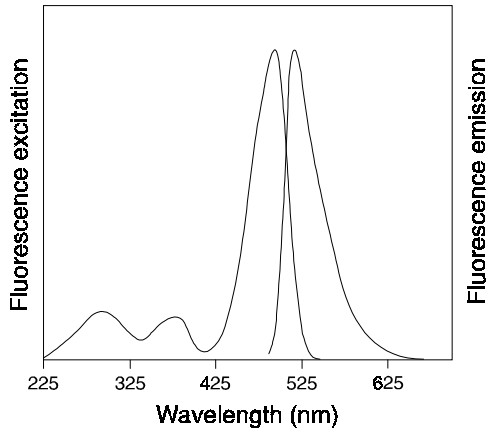

Figure 1. *Fluorescence excitation and emission spectra of dsDNA-bound SYBR Green I nucleic acid gel stain.*

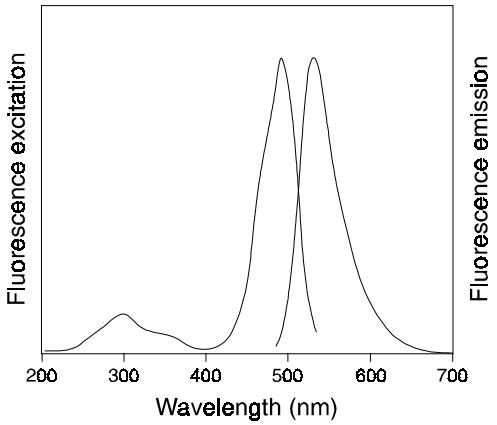

Figure 2. *Excitation and emission spectra of SYBR Gold nucleic acid gel stain bound to double-stranded DNA.*

SYBR dyes. One consideration, however, is the intensity of the light provided by your transilluminator. Many older transilluminators use four or six 8-watt tubes. The Fotodyne Foto/UV® 450 transilluminator that we use has six 15-watt tubes and consequently provides much more intense illumination.

6.3 Epi-Illuminators

Greater sensitivity can be obtained with SYBR stains by positioning the excitation light source above the gel, known as "epi-illumination" (analogous to epi-fluorescence microscopy). Epi-illumination results in lower background levels from the gel and also eliminates the problem of having the light source pointing directly into the camera. The trade-off is that epi-illumination requires much longer exposure times. Handheld UV lamps are generally not powerful enough for photography, though they can be useful for monitoring the progress of bands during electrophoresis. When held very close to the gel, handlamps can be used to see bands that contain more than about 1 ng of DNA.

6.4 Photographic Film Type

Photographs should be taken with a high-speed film, such as Polaroid® 667 black-and-white print film (ISO 3000). Many of the other Polaroid films, particularly the 665 positive/negative film, which gives both prints and negatives, are much slower (ISO 50–100). Slower films require very long exposure times resulting in bleaching of the signal. In addition to the Polaroid 667 print film, it has been reported that KODAK® T-MAX P3200 35 mm black-and-white negative film also gives good results. If it is necessary to have a negative for publication, we have found that prints made with Polaroid 667 sheet film can be photographed onto black-and-white 35 mm negative film easily, with excellent results.

6.5 The SYBR Green Stain Photographic Filter

SYBR stains emit maximally around 520–550 nm, so it is vital to use a filter that is optimal for these wavelengths. Typical "ethidium bromide filters" block green light and will not give good results with SYBR gel stains. The SYBR Green/Gold gel stain photographic filter (S-7569) we offer is a cut-off filter that blocks light below 500 nm. The filter is a flexible "gelatin" type filter that is 75 × 75 mm (3 × 3 inches) square. This can be cut to fit any shape or size of holder. If you do not have a holder attached to your camera you can use tape to hold the filter in position in front of your lens. Also, given the somewhat delicate nature of these filters, it is advisable to place them in a protective frame.

6.6 Fogging and Increased Background in Photographs

It is desirable, but not essential, to use a UV-blocking filter in conjunction with the SYBR Green/Gold gel stain photographic filter. The SYBR Green/Gold gel stain photographic filter has a narrow window of low transmittance in the 300 nm region (where most transilluminators emit) and this can result in "fogging" or increased background in the photograph. A UV-blocking 2A or 2B Wratten® filter (or equivalent) will help block these wavelengths and reduce background light. A UV-blocking filter may be important if your transilluminator light is especially intense.

6.7 Photographic Filter Alternatives

Standard photographic glass filters can also be used and are obtainable at many photographic supply stores. Select a filter that has a cut off in the 500–510 nm range for optimal results; such a filter will appear yellow or deep yellow in color. These filters can usually be screwed directly into the end of the camera lens. If the filter you select transmits UV light, be sure to also use a UV-blocking filter. The appearance of even faint images of the illuminator bulbs in your photos is a strong indicator that the filter is allowing UV light to pass.

6.8 Photographic Filter Combinations

Do not use the SYBR gel stain photographic filter in conjunction with the standard red filter that most people use for ethidium bromide. Such a combination will have the same effect as using the ethidium bromide filter alone, effectively blocking the largest part of the light emitted from the SYBR stain.

6.9 Blue-Light Transilluminators

We have found that blue-light transilluminators, such as the Clare Chemical Dark Reader™ provide the same sensitivity as UV transilluminators, without the risk of UV exposure. For optimal sensitivity, use the filters recommended by the manufacturer.

6.10 Gel Scanners

Instruments such as Hitachi's FMBIO® and Molecular Dynamics' FluorImager™ and Storm™ systems employ laser scanning technology to visualize gels. The SYBR stains perform excellently with these types of machines, although the SYBR

Gold stain gives somewhat higher background. In these instruments the level of detection per band, for DNA using SYBR Green I nucleic acid stain, is in the picogram range.[2]

6.11 Video Camera Sensitivity

Video and CCD cameras are becoming increasingly popular as methods of gel documentation. However, the spectral response of most video and CCD cameras is very different from photographic film. Our sensitivity claims for SYBR stains are based on photography using Polaroid 667 black-and-white print film. If you use a video documentation system, you might not achieve the same sensitivity levels. However, you will still find SYBR stains to be more sensitive than ethidium bromide, especially for the detection of RNA or small DNA fragments.

6.12 Filters for Video Cameras

Some video camera systems come supplied with glass optical filters. To be certain that one of these will work with SYBR stains we suggest you contact the instrument manufacturer and obtain the spectral data on the filters. Most CCD cameras are much less sensitive to UV light than is Polaroid 667 black-and-white print film. Consequently, it is generally not necessary for the filter to block all light transmittance in the UV range. However, most CCD cameras are quite sensitive to infrared (IR) light and require an IR-blocking filter to prevent the camera from imaging the IR emissions from the transilluminator lamps. Replacing the manufacturer's filter with a filter not designed for CCD cameras may result in the appearance of high background from the lamps.

References

1. Mutat Res 439, 37 (1999); **2.** Anal Biochem 239, 223 (1996).

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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