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Product Information

SYBR® Green I nucleic acid gel stain

Catalog Number **S9430**
Storage Temperature –20 °C

CAS RN 163795-75-3

Product Description

SYBR Green I is an ultrasensitive stain for post-electrophoresis staining of double stranded DNA (dsDNA) in agarose or polyacrylamide gels. The detection limit is ~60 pg per band with 300 nm transillumination (down to 20 pg with 254 nm epi-Illumination).[1,2] This sensitivity is 25 times greater than can be achieved with ethidium bromide using standard 300 nm transillumination. SYBR Green I can also be used to detect single-stranded DNA and RNA in denaturing agarose/formaldehyde and polyacrylamide/urea gels without any prewashing steps, although the sensitivity is lower (100–300 pg per band using 254 nm epi-illumination).

SYBR Green I is also a very sensitive stain for oligonucleotides, allowing for detection of as little as 1–2 ng of a synthetic 24-mer on a 5% polyacrylamide gel, which is 50–100 times greater sensitivity than obtained with ethidium bromide.[2,3] Staining agarose gels with SYBR Green I does not interfere with the transfer of nucleic acids to membranes or subsequent hybridization in Southern or Northern blot analysis as long as 0.1–0.3% SDS is included in prehybridization and hybridization buffers to remove the dye.

The fluorescence quantum yield of the DNA/SYBR Green I complex (~0.8) is over five times greater than that of the DNA/ethidium bromide complex (~0.15). The maximum excitation wavelength of SYBR Green I is 497 nm, but there is also a secondary excitation peak near 254 nm. The fluorescence emission of SYBR Green I stained DNA is centered at 520 nm.

The exceptional sensitivity of SYBR Green I makes it useful for many applications with a limited amount of DNA. This sensitivity may allow the replacement of radioisotopes in many applications. Because of the strong DNA binding affinity of SYBR Green I, it can be used to stain DNA before electrophoresis (pre-staining), as well as after electrophoresis (post-staining).[4,5] Gels can be precast with SYBR Green I; however, the greatest sensitivity is achieved with post-staining.

The binding of SYBR Green I to DNA does not inhibit the activity of many common restriction endonucleases, including *Hin*d III and *Eco*R I.[1,2] SYBR Green I does not need to be removed for in-gel digestion and ligation techniques.[6]

Component

The dye is supplied as a 10,000× solution in dimethyl sulfoxide (DMSO). One ml of solution prepares 10 liters of Staining Solution, sufficient for 100 mini gels.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Before opening, allow the product to warm to room temperature and then briefly centrifuge to collect at the bottom of the vial. The Staining Solution should be prepared in a plastic rather than a glass container, as the stain may adsorb to glass surfaces.

Prepare the Staining Solution with a 1:10,000 dilution of the product in **one** of the following buffers:

- 1× TE buffer (10 mM Tris-HCl with 1 mM EDTA, pH 8.0)
- 1× TBE buffer (89 mM Tris base, 89 mM boric acid, and 1 mM EDTA, pH 8.0)
- 1× TAE buffer (40 mM Tris-acetate with 1 mM EDTA, pH 8.0)

Note: The buffer should not have been used previously for electrophoresis for optimal results. For optimal sensitivity, verify that the pH is between 7.5–8.0 (preferably pH 8.0) as SYBR Green I is pH sensitive.

Storage/Stability

Store the product at –20 °C. The diluted Staining Solution may be stored protected from light either at 2–8 °C for several weeks or at room temperature for 3–4 days. Staining Solution prepared in water is less stable than those prepared in buffer and must be used within 24 hours.

Procedure

Staining Procedure

Perform electrophoresis according to standard procedures.[7] Perform staining at room temperature and protect the Staining Solution from light.

1. 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, as the fluorescence of SYBR Green I complexes is not quenched by urea nor formaldehyde.

2. 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.

   Notes: The staining solution may be stored at 2–8 °C in the dark and reused three to four times.

   SYBR Green I does not interfere with the transfer of DNA/RNA to membranes or subsequent hybridization in Southern or Northern blot analysis as long as 0.1–0.3% SDS is included in prehybridization and hybridization buffers to remove the dye.

   SYBR Green I may be removed from dsDNA by ethanol precipitation.

   Dilute Staining Solutions of SYBR Green I may be poured through activated charcoal. One gram of activated charcoal can absorb the dye from 10 liters of freshly prepared Staining 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).

   Note: SYBR Green I has low intrinsic fluorescence, so there is no need to remove free dye. The stained gels have negligible background fluorescence.

2. The gel may be photographed with black and white print film using a Wratten 15 filter (Catalog Number F8390). If the transilluminator light is especially intense, a UV-blocking Wratten 2A filter (Catalog Number F9265) may be used in conjunction with the Wratten 15 filter to prevent “fogging” or increased background in the photograph. A 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 use with SYBR Green I.

   Notes: The negligible background fluorescence allows long film exposures when detecting small amounts of DNA. 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.

   Video and CCD cameras in general have a different spectral response than black and white print film, and may not exhibit the same sensitivity.

References

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2. *Biomedical Products*, **19**, 68 (1994).
3. *BioProbes*, **20**, 12 (1994).
4. *Methods Enzymol.*, **217**, 414 (1993).
5. *Nucleic Acids Res.*, **20**, 2803 (1992).
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7. Sambrook, J. and Russell, D.W., Molecular Cloning: A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory Press (Cold Spring Harbor, New York: 2001).

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