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Product Information

SYBR® Green II RNA gel stain

Catalog Number **S9305**
Storage Temperature –20 °C

CAS RN 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. 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).[1]

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 may be used with commonly available ultraviolet epi- and transilluminator excitation sources, as well as hand-held ultraviolet lamps.

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).[2,3]

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 of RNA per band in agarose/formaldehyde gels or polyacrylamide/urea gels using 254 nm epi-illumination, and ~4 ng of RNA per band using 300 nm transillumination. 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 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,[4] that require extremely sensitive detection techniques.

Conventional SSCP analysis requires radioactive hybridization probes. Although nonisotopic techniques for SSCP analysis have been developed,[5-8] 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.[9] 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.

Components
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 in 1× TBE buffer (89 mM Tris base, with 89 mM boric acid and 1 mM EDTA, pH 8.0). For denaturing agarose/ formaldehyde gels, make a 1:5,000 dilution in 1× TBE.

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 II 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:
Perform electrophoresis according to standard procedures.[10] Perform staining at room temperature and protect 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 II complexes is not quenched by urea or 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 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.

   Dilute Staining 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 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 II has a low intrinisic 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 visualizing SYBR Green II stained gels as they give poor results.

   Notes: The negligible background fluorescence, allows 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.

   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

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