Lumiprobe
Life science solutions

RNA Staining in Gels with ssGreen®

ssGreen® is one of the most sensitive stains for post-electrophoresis staining of RNA and single-stranded DNA (ssDNA) in agarose or polyacrylamide gels. The fluorescence quantum yield of the ssGreen/RNA complex is more than 7× higher than that of the ethidium bromide/RNA complex. Although ssGreen is not selective for RNA staining, the dye exhibits about 1.5× greater quantum yield when bound to RNA than double-stranded DNA, which makes it unique among all nucleic acid dyes.

ssGreen staining is compatible with denaturing gels. On agarose/formaldehyde and polyacrylamide/urea gels, the sensitivity of ssGreen is slightly reduced but still superior to that of ethidium bromide. Staining agarose/formaldehyde gels with ssGreen does not interfere with transfer of RNA to filters or subsequent hybridization in Northern blot analysis as long as 0.1%–0.3% SDS is included in prehybridization and hybridization buffers.

Staining gels with ssGreen has fewer steps than those with ethidium bromide. Because the fluorescence of ssGreen/RNA complexes is not quenched by formaldehyde or urea, there is no need to wash these denaturants out of gels before staining. Also, ssGreen stain has a low intrinsic fluorescence, allowing gel viewing and photographing without preliminary removing unbound dye.

Handling and Disposal

Before opening, each vial should be warm to room temperature and then briefly centrifuged in a microfuge to drop the ssGreen solution at the bottom of the vial.

Please note that there is currently no data on the mutagenicity or toxicity of ssGreen. Since this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with care. The DMSO stock solution requires special attention, as DMSO can enhance the absorption of organic molecules into tissues. We strongly recommend wearing double gloves when handling the DMSO stock solution. As with all nucleic acid reagents, ssGreen solutions should be disposed of in accordance with local regulations.

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Staining RNA

1. Perform electrophoresis on non-denaturing gels or denaturing polyacrylamide/urea or agarose/formaldehyde gels according to standard techniques.
2. Dilute the stock ssGreen solution. To stain a 20 mL agarose gel, 35–50 mL of dye solution will be sufficient. For non-denaturing gels and denaturing polyacrylamide/urea gels, use a 1:10,000 dilution in 1× TBE. For denaturing agarose/formaldehyde gels, use a 1:5000 dilution in 1× TBE. Mix thoroughly.

   Important! Staining with ssGreen is pH sensitive. For best results, the staining solution pH should be between 7.5 and 8.0 (preferably pH 8.0) at the temperature used for staining.
3. Place the gel in a plastic tray (tip box lid or household storage container).

   Important! Do not use a glass container, as the dye may adsorb to the walls of the container, resulting in poor gel staining.
4. Add enough dye solution volume to cover the gel.
5. Cover the gel and the staining solution with aluminum foil or place them in the dark to protect from light.
6. Agitate the gel gently at room temperature. The optimal staining time is typically 20–40 minutes for agarose gels and 10–40 minutes for polyacrylamide gels. The staining time may vary depending on the thickness of the gel and the percentage of agarose or polyacrylamide.
7. The staining solution may be stored at 4 °C in the dark and reused three to four times.

Staining DNA

ssGreen is not selective to RNA dye and can be used to stain DNA in gels. Use our protocol for DNA staining in gels with dsGreen®.

Note that adding ssGreen dye directly to molten agarose in a ratio of 1:10,000 changes the mobility of fragments; therefore, for optimal separation of fragments in the gel, increasing the electrophoresis time by 1.5-2 times is necessary.

Viewing and photographing gels

ssGreen is maximally excited at 483 nm but has a secondary excitation peak centered near 254 nm (not shown). The fluorescence emission of the ssGreen/RNA complex is centered at 518 nm.

View or document the gel using an available light source and a green/yellow filter. Transilluminators with blue light or UV low-pressure mercury lamps (254 nm) can be used to visualize gels stained with ssGreen. A high-pressure mercury lamp (365 nm) can be used, too, but this light source gives somewhat less efficient excitation.

Stained gels have negligible background fluorescence, allowing long exposures (up to 2 minutes) to detect small amounts of RNA.

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