Nucleic Acid Staining in Gels with dsGold®

dsGold, or Oxazole Gold, is an asymmetrical cyanine dye used to stain dsDNA, ssDNA, and RNA in electrophoretic gels.

dsGold exhibits more than 1000-fold fluorescence enhancement upon binding to nucleic acids and has the highest quantum yield (0.6–0.7) of the dye-nucleic acid complexes compared to other stains such as ethidium bromide (EtBr), dsGreen, and ssGreen. The dye-nucleic acid complexes have two fluorescence excitation maxima, at ~300 nm and ~495 nm, and a single emission maximum at ~546 nm. Thus, dsGold-stained gels can be visualized using UV and blue light transilluminators with appropriate filters.

dsGold allows the detection of as little as 25 pg of DNA in denaturing urea, glyoxal, and formaldehyde gels. The stain is able to penetrate thick and high-percentage agarose gels rapidly. Due to the low fluorescence of the unbound dye, formaldehyde agarose gels do not require the destaining procedure. The presence of dsGold in stained gels at standard working concentrations does not interfere with T4 DNA ligase, Taq polymerase, restriction endonucleases, or Northern or Southern blotting. The dye can be easily removed from nucleic acids by ethanol precipitation, leaving pure templates available for subsequent manipulation or analysis.

Handling and Disposal

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

Please note that there is currently no data on the mutagenicity or toxicity of dsGold. 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, dsGold solutions should be disposed of in accordance with local regulations.

Nucleic acid staining

This protocol describes procedures for staining nucleic acids by soaking the gel in a dsGold dye solution. Adding the dye to the gel prior to electrophoresis is not recommended, as dsGold causes a substantial slowdown in the electrophoretic mobility of nucleic acids in the gel.

1. Transfer the required 1× TAE, TE, or TBE volume into a plastic container. For a 20 mL agarose gel, 35–50 mL of dye solution is sufficient.
2. Add dye concentrate to the buffer at a ratio of 1:10,000. Mix thoroughly.

   Important! dsGold staining is pH sensitive. For best results, the pH of the staining solution should be between 7.5 and 8.0 (preferably pH 8.0) at the temperature used for staining.
3. Run the sample(s) on an agarose or polyacrylamide gel. Pre-washing gels, including those containing urea, formaldehyde, or glyoxal, is not required.

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4. Place the gel in a plastic tray (tip box lid or household food storage container).

   Important! Do not use a glass container; the dye may adsorb to the walls, resulting in insufficient gel staining.

5. Add enough volume of staining solution to cover the gel completely.
6. Cover the gel and staining solution with aluminum foil or keep them in a dark place to protect them from light.
7. Incubate the gel in the staining solution for at least 20 minutes at 37 °C with constant shaking on an orbital shaker at 50–80 rpm. Incubation time depends on the thickness of the gel and the percentage of agarose or polyacrylamide.
8. The staining solution can be stored in the dark and reused 3–4 times, but we recommend using the fresh staining solution for the best results.

Viewing and photographing the gel

dsGold is maximally excited at 495 nm. The fluorescence emission of the dsGold/RNA complex is centered at 546 nm.

View or document the gel using an available light source and a green/yellow filter. Optimal exposure time or other instrument settings are determined empirically.

Blue light transilluminators or 254 and 300 nm UV mercury lamps can be used to visualize dsGold-stained gels. Stained gels can also be visualized and analyzed using laser scanners.

Removal of dsGold stain from nucleic acids

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

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 M ammonium acetate. Mix gently.
2. Add two volumes of ice-cold absolute ethanol to the sample and mix well. Incubate at 0 °C (on ice) for 30 minutes.
3. Precipitate nucleic acids by centrifugation for at least 15 minutes at 10,000–12,000 × *g*.
4. Discard the supernatant and wash the pellet with 70% ethanol.
5. Repeat centrifugation to precipitate nucleic acids.
6. Allow the pellet to air dry and resuspend if necessary.

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