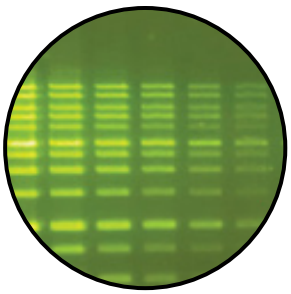
PROTOCOL

Green DNA Fluorescent Stain



Part Number:	GMD-500
Version:	1.0
Storage:	Stable for up to 1 year at -20°C.
Batch Number:	Marked on tube

Size: 500 ul (10,000X in DMSO)
Working Reagent Preparation: 1:10,000 dilution in TE, TAE or TBE buffer
Contents: Fluorescent Dye in dimethyl sulfoxide with the 10,000X concentration

Protocol

Post-Electrophoresis DNA Staining

1. Perform electrophoresis on an agarose gel
   - The Green DNA is compatible TAE (40mM Tris-acetate, 1mM EDTA, pH 8), TBE (89mM Tris base, 89mM boric acid, 1mM EDTA, pH 8), and TE (20mM Tris base, 1mM EDTA, pH 8) buffers.
2. Dilute the stock Green DNA reagent with the 1:10,000 ratio.
   - Stock stain can be diluted in the TE, TAE or TBE buffer.
   - If the staining solution is diluted in water, it should be used within 24 hours. The buffered solution may increase the stability for this fluorescent staining dye.
3. Cover the gel with the staining solution and incubate at the room temperature for 10-30 minutes.
   - Use a plastic container. Do not use a glass container since it will adsorb much of the dye in the staining solution.
   - Protect the staining container from light by covering it with the aluminum foil or place it in the dark.
   - Agitate the gel gently at the room temperature.
   - Staining time will vary with the thickness of the gel and the agarose percentage.
   - No destaining is required.
   - The staining solution may be stored in the dark and at the low temperature for a week or more.
4. Photograph the gel with UV or blue-light transilluminator.
   - It is important to clean the surface of the transillumuntor after/before each use with the deionized water and a soft cloth. Otherwise, fluorescent dyes may accumulate on the glass surface and cause a high fluorescent background.
   - Video cameras and CCD cameras have a spectral response different from the black-and-white print film, thus it may not exhibit the same degree of sensitivity.

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Precasting Green DNA Gels

The Green DNA can also be applied to the precast agarose gels. This fluorescent dye stock solution is diluted by 1:10,000 in the gel solution just prior to pouring the gel. However, the DNA detection limit for the precast agarose gel with the Green DNA may be slightly higher than the gel stained after electrophoresis. In addition, the migration rate of DNA fragments in the Green DNA gels may be significantly slower than that for the same fragments in a gel without the fluorescent dye. We therefore would not suggest to stain the precast gel with the Green DNA based on our experiences as described above.

Troubleshooting

Problem	Cause	Solution
Low Sensitivity	Wavelength may not be right	Check the fluorescence excitation and emission wavelengths.
	Dilution ratio may not be right	Check the dilution ratio in the 10,000- fold dilution.

Caution

• Before opening, the vial should be warmed completely to the ambient temperature for ensuring that the DMSO is thawed thoroughly and that the solution is homogeneous.

• The DMSO stock solution should be handled with applicable caution because DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of the stain in compliance with local regulations.

• There is no data addressing the mutagenicity or toxicity of the fluorescent dye in humans.
However, the fluorescent dye binds to nucleic acids; it should be recognized as a potential mutagen and used with appropriate care.

• Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.

Spectral Characteristics

Green DNA is ecited at 497 nm but also whos a secondary excitation peak at 248 nm (Fig 1a). After bound to DNA, the fluorescent emission of the Green DNA is centered at 524 nm (Fig 1b). These sepctral characteristics enable this fluorescent dye to be compatible with a wide variety of gel reading facilities, including UV epi- and transilluminator, argon laser and mercury-arc lamp excitation gel scanners.

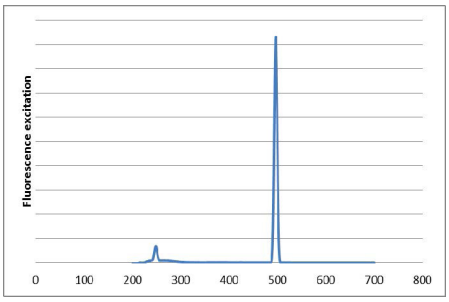

Fig 1a Fluorescent excitation spectra of the Green DNA

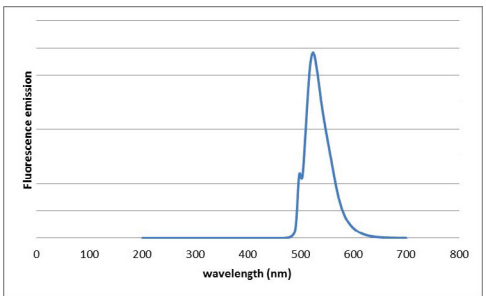

Fig 1b Fluorescence excitation and emission spectra of the Green DNA bound to dsDNA